It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to PENNIE & EDMONDS LLP Deposit Account No. 16-1150.

AMENDMENTS

Prior to examination on the merits, please consider the following amendments and remarks.

IN THE SPECIFICATION

A marked up version of the specification showing the amendments is attached hereto as Exhibit A.

Please amend the specification as follows:

Page 11, lines 16 through 24:

4. DESCRIPTION OF THE FIGURES

FIG.1. In vivo cholesterol differential display. mRNA prepared from human monocytes isolated from the blood of patients on different diets. cDNA prepared from one patient on a high fat diet/high serum cholesterol (lanes 1,2) and low fat diet/low serum cholesterol (lanes 3,4) was displayed using the forward primer $T_{11}XG$ (SEQ ID NO. 8) and the reverse primer OPO14 (agcatggctc)(SEQ ID NO. 9). The DNA corresponding to marked band (#14) was excised and amplified for sequence analysis.

Page 11, line 31 through page 12, line 28:

FIG.3. Quantitative RT-PCR analysis of mouse bcl-2 mRNA levels in apoE-deficient mice. Monocyte RNA from apoE-deficient and control mice was compared using primers for mouse bcl-2 (for-cacccctggcatcttctccttcc (SEQ ID NO. 10) /rev-atcctccccccagttcaccccatcc) (SEQ ID NO. 11) shown in the upper panel and mouse γActin (for-cctgatagatgggcactgtgt (SEQ ID NO. 12)/rev-gaacacggcattgtcactaact (SEQ ID NO. 13)) shown in the lower panel. A 1:3 dilution series of each input cDNA was done in pairs with the left band in each pair deriving from wild-type cDNA and the right band from apoE-deficient cDNA.

FIG.4. RT-PCR quantification of human glutathione peroxidase (HUMGPXP1) cDNA from human clinical samples cDNA prepared from RNA derived from blood monocytes of the same patient under a high fat diet (serum cholesterol level = 200; top panel) and a low fat diet (serum cholesterol level = 170; bottom panel). Dilution series of amplification products using GPX1.3 primers derived from HUMGPXP1 sequences 1121-1142 (for-aagtcgcgcccgccccctgaaat) (SEQ ID NO. 14) and 1260-1237 (rev-gatccctggccaccgtccgtctga)(SEQ ID NO. 15) is shown in the left portion of each panel. Dilution series of amplification products using human actin primers (for-accctgaagtaccccat (SEQ ID NO. 16)/rev-tagaagcatttgcggtg)(SEQ ID NO. 17) is shown in the right portion of each panel. The HUMGPXP1 band decreased in intensity under a high fat diet (compare top left to bottom left), whereas the actin control band was equally intense under each diet (compare top right to bottom right).

FIG.5. IL-1 activated HUVEC differential display. mRNA prepared from control HUVEC (lanes 9,10), 1 hr. of 10 units/ml IL-1 treatment (lanes 7,8), or 6 hr. treatment (lanes 11,12), was used in differential display reactions with the forward primer OPE7 (agatgcagcc) (SEQ ID NO. 18) and reverse primer T_{11}XA (SEQ ID NO. 19), which is an equimolar mix of

oligonucleotides where X is G, C, or A. The DNA corresponding to marked band, rchd005, was excised and amplified for Northern analysis and subcloning.

Page 13, lines 1 through 13:

FIG.8. Band rchd005 DNA sequence (SEQ ID NO. 1). The sequence was determined by sequencing the insert of pRCHD005, resulting from the ligation of amplified rchd005 sequences into the TA cloning vector.

FIG.9. IL-1 activated HUVEC differential display. mRNA prepared from control HUVEC (lanes 3,4), 1 hr. of 10 units/ml IL-1 treatment (lanes 1,2), or 6 hr. treatment (lanes 5,6), was used in differential display reactions with the forward primer OPG20 (tctcccctcag)(SEQ ID NO. 20) and reverse primer $T_{11}XC$ (SEQ ID NO. 21), which is an equimolar mix of oligonucleotides where X is G, C, or A. The DNA corresponding to marked band, rchd024, was excised and amplified for Northern analysis and subcloning.

Page 13, line 25 through page 14, line 23:

FIG.12. Band rchd024 DNA sequence (SEQ ID NO. 2). The sequence was determined by sequencing the insert of pRCHD024, resulting from the ligation of amplified rchd024 sequences into the TA cloning vector.

FIG.13. IL-1 activated HUVEC differential display for rchd032. mRNA prepared from control HUVEC (lanes 3,4), 1 hr. of 10 units/ml IL-1 treatment (lanes 1,2) , or 6 hr. treatment (lanes 5,6), was used in differential display reactions with the forward primer OPI9 (tggagagcag)(SEQ ID NO. 22) and reverse primer $T_{11}XA$, which is an equimolar mix of oligonucleotides where X is G, C, or A. The DNA corresponding to marked band, rchd032, was excised and amplified for Northern analysis and subcloning.

FIG.14. RT-PCR quantification of rchd032 cDNA from IL-1 activated HUVEC's cDNA prepared from RNA derived from control, 1hr., and 6 hr. IL-1 activated HUVEC's. Shown in lanes 1,2, and 3 are a 5 fold dilution series of input cDNA amplified in the upper panel with rchd032 primers (for-atttataaaggggtaattcatta)(SEQ ID NO. 23)/rev-ttaaagccaatttcaaaataat)(SEQ ID NO. 24)), and in the lower panel with human actin primers (for-accctgaagtaccccat/rev-tagaagcatttgcggtg). A band at the 1:125 dilution in lane 3 is visible in the 6 hr. sample but not in the control.

FIG.15. Band rchd032 DNA sequence (SEQ ID NO. 3). The sequence was determined by sequencing the insert of pRCHD032, resulting from the ligation of amplified rchd032 sequences into the TA cloning vector.

FIG.16. IL-1 activated HUVEC differential display for rchd036. mRNA prepared from control HUVEC (lanes 3,4), 1 hr. of 10 units/ml IL-1 treatment (lanes 1,2), or 6 hr. treatment (lanes 5,6), was used in differential display reactions with the forward primer OPI17 (ggtggtgatg) (SEQ ID NO. 25) and reverse primer $T_{11}XC$, which is an equimolar mix of oligonucleotides where X is G, C, or A. The DNA corresponding to marked band, rchd036, was excised and amplified for Northern analysis and subcloning.

Page 14, lines 31 through 34:

FIG.18. Band rchd036 DNA sequence (SEQ ID NO. 4). The sequence was determined by sequencing the insert of pRCHD036, resulting from the ligation of amplified rchd036 sequences into the TA cloning vector.

Page 15, lines 20 through 32:

FIG.22. Band rchd502 DNA sequence (SEQ ID NO. 5). The sequence was determined by sequencing the insert of pRCHD502, resulting from the ligation of amplified rchd502 sequences into the TA cloning vector.

FIG.23. Laminar shear stress HUVEC differential display for rchd505. mRNA prepared from control HUVEC (lanes 3,4), 1 hr. (lanes 1,2) or 6 hr. (lanes 5,6) of 10 dyn/cm2 laminar shear stress treatment was used in differential display reactions with the forward primer OPE2 (ggtgcgggaa) (SEQ ID NO. 26) and reverse primer T_{11}XA, which is an equimolar mix of oligonucleotides where X is G,C, or A. The DNA corresponding to marked band, rchd505, was excised and amplified for Northern analysis and subcloning.

Page 16, line 9 through Page 17, line 2:

FIG.26. Laminar shear stress HUVEC differential display for rchd523. mRNA prepared from control HUVEC (lanes 3,4), 1 hr. (lanes 1,2) or 6 hr. (lanes 5,6) of 10 dyn/cm2 laminar shear stress treatment was used in differential display reactions with the forward primer OPI11 (acatgccgtg) (SEQ ID NO. 27) and reverse primer T_{11}XC, which is an equimolar mix of oligonucleotides where X is G,C, or A. The DNA corresponding to marked band, rchd523, was excised and amplified for Northern analysis and subcloning.

FIG.27. RT-PCR quantification of rchd523 cDNA from shear stressed endothelial cell cDNA prepared from RNA derived from control, 1hr., and 6 hr. shear stressed HUVEC's. Shown in lanes 1,2, and 3 are a 5-fold dilution series of input cDNA amplified in the upper panel with rchd523 primers (for-atgccgtgtgggttagtc (SEQ ID NO. 28)/rev-attttatgggaaggtttttaca) (SEQ ID NO. 29), and in lanes 4 and 5, a 5-fold dilution series using

human actin primers (for-accctgaagtaccccat/rev-tagaagcatttgcggtg). A band at the 1:5 dilution in lane 2 is visible in the 6 hr. sample but not in the control.

FIG.28. DNA (SEQ ID NO. 6) and encoded amino acid (SEQ ID NO. 38) sequence of the rchd523 gene.

FIG.29. Laminar shear stress HUVEC differential display for rchd528. mRNA prepared from control HUVEC (lanes 3,4), 1 hr. (lanes 1,2) or 6 hr. (lanes 5,6) of 10 dyn/cm2 laminar shear stress treatment was used in differential display reactions with the forward primer OPI19 (aatgcgggag) (SEQ ID NO. 30) and reverse primer $T_{11}XG$, which is an equimolar mix of oligonucleotides where X is G,C, or A. The DNA corresponding to marked band, rchd528, was excised and amplified for Northern analysis and subcloning.

Page 17, lines 10 through 13:

FIG.31. Band rchd528 DNA sequence (SEQ ID NO. 7). The sequence was determined by sequencing the insert of pRCHD528, resulting from the ligation of amplified rchd528 sequences into the TA cloning vector.

Page 17, lines 25 through 26:

FIG.35. DNA (SEQ ID NO. 36) and encoded amino acid (SEQ ID NO. 37) sequence of the rchd534 gene.

Page 38, lines 19 through 24:

5.4.1. DIFFERENTIALLY EXPRESSED AND PATHWAY GENE SEQUENCES

The differentially expressed and pathway genes of the invention are listed below, in Table 1. Differentially expressed and pathway gene nucleotide sequences are shown in FIGS.

8 (SEQ ID NO. 1), 12 (SEQ ID NO. 2), 15 (SEQ ID NO. 3), 18 (SEQ ID NO. 4), 22 (SEQ ID NO. 5), 28 (SEQ ID NO. 38), 31 (SEQ ID NO. 7), and 35 (SEQ ID NOS. 36-37).

Page 39, line 19 through page 40, line 2:

The genes listed in Table 1 may be obtained using cloning methods well known to those skilled in the art, including but not limited to the use of appropriate probes to detect the genes within an appropriate cDNA or gDNA (genomic DNA) library. (See, for example, Sambrook et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratories, which is incorporated by reference herein in its entirety). Probes for the novel sequences reported herein may be obtained directly from the isolated clones deposited with the NRRL, as indicated in Table 2, below. Alternatively, oligonucleotide probes for the novel genes may be synthesized based on the DNA sequences disclosed herein in FIGs.8 (SEQ ID NO. 1), 12 (SEQ ID NO. 2), 15 (SEQ ID NO. 3), 18 (SEQ ID NO. 4), 22 (SEQ ID NO. 5), 28 (SEQ ID NO. 38), 31 (SEQ ID NO. 7), and 35 (SEQ ID NOS. 36-37). Such synthetic oligonucleotides may be similarly produced based on the sequences provided for the previously known genes described in the following references: Cleary et al., 1986, Cell 47: 19-28 (bcl-2); Takahashi et al., 1990, J. Biochem 108: 145-148 (glutathione peroxidase); and Jones et al., 1993, J. Biol. Chem. 268: 9049-9054 (prostaglandin endoperoxide synthase II), each of which is incorporated herein in its entirety.

Page 43, line 33 through Page 44, line 35:

As used herein, "differentially expressed gene" (i.e. target and fingerprint gene) or "pathway gene" refers to (a) a gene containing at least one of the DNA sequences disclosed herein (as shown in FIGS. 8 (SEQ ID NO. 1), 12 (SEQ ID NO. 2), 15 (SEQ ID NO. 3), 18

(SEQ ID NO. 4), 22 (SEQ ID NO. 5), 28 (SEQ ID NO. 38), 31 (SEQ ID NO. 7), and 35 (SEQ ID NOS. 36-37)), or contained in the clones listed in Table 2, as deposited with the NRRL; (b) any DNA sequence that encodes the amino acid sequence encoded by the DNA sequences disclosed herein (as shown in FIGS. 8 (SEQ ID NO. 1), 12 (SEQ ID NO. 2), 15 (SEQ ID NO. 3), 18 (SEQ ID NO. 4), 22 (SEQ ID NO. 5), 28 (SEQ ID NO. 38), 31 (SEQ ID NO. 7), and 35 (SEQ ID NOS. 36-37)), contained in the clones, listed in Table 2, as deposited with the NRRL or contained within the coding region of the gene to which the DNA sequences disclosed herein (as shown in FIGS. 8 (SEQ ID NO. 1), 12 (SEQ ID NO. 2), 15 (SEQ ID NO. 3), 18 (SEQ ID NO. 4), 22 (SEQ ID NO. 5), 28 (SEQ ID NO. 38), 31 (SEQ ID NO. 7), and 35 (SEQ ID NOS. 36-37)) or contained in the clones listed in Table 2, as deposited with the NRRL, belong; (c) any DNA sequence that hybridizes to the complement of the coding sequences disclosed herein, contained in the clones listed in Table 2, as deposited with the NRRL, or contained within the coding region of the gene to which the DNA sequences disclosed herein (as shown in FIGS. 8 (SEQ ID NO. 1), 12 (SEQ ID NO. 2), 15 (SEQ ID NO. 3), 18 (SEQ ID NO. 4), 22 (SEQ ID NO. 5), 28 (SEQ ID NO. 38), 31 (SEQ ID NO. 7), and 35 (SEQ ID NOS. 36-37)) or contained in the clones listed in Table 2, as deposited with the NRRL, belong, under highly stringent conditions, e.g., hybridization to filter-bound DNA in 0.5 M $NaHPO_4$, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1xSSC/0.1% SDS at 68°C (Ausubel F.M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing Associates, Inc., and John Wiley & sons, Inc., New York, at p. 2.10.3) and encodes a gene product functionally equivalent to a gene product encoded by sequences contained within the clones listed in Table 2; and/or (d) any DNA sequence that hybridizes to the complement of the coding sequences disclosed herein, (as shown in FIGS. 8 (SEQ ID NO. 1), 12 (SEQ ID NO. 2), 15 (SEQ ID NO. 3), 18 (SEQ ID NO. 4), 22 (SEQ ID

NO. 5), 28 (SEQ ID NO. 38), 31 (SEQ ID NO. 7), and 35 (SEQ ID NOS. 36-37)) contained in the clones listed in Table 2, as deposited with the NRRL or contained within the coding region of the gene to which DNA sequences disclosed herein (as shown in FIGS. 8 (SEQ ID NO. 1), 12 (SEQ ID NO. 2), 15 (SEQ ID NO. 3), 18 (SEQ ID NO. 4), 22 (SEQ ID NO. 5), 28 (SEQ ID NO. 38), 31 (SEQ ID NO. 7), and 35 (SEQ ID NOS. 36-37)) or contained in the clones, listed in Table 2, as deposited with the NRRL, belong, under less stringent conditions, such as moderately stringent conditions, e.g., washing in 0.2xSSC/0.1% SDS at 42°C (Ausubel et al., 1989, *supra*), yet which still encodes a functionally equivalent gene product.

Page 49, lines 1 through 14:

5.4.2. DIFFERENTIALLY EXPRESSED AND PATHWAY GENE PRODUCTS

Differentially expressed and pathway gene products include those proteins encoded by the differentially expressed and pathway gene sequences described in Section 5.4.1, above. Specifically, differentially expressed and pathway gene products may include differentially expressed and pathway gene polypeptides encoded by the differentially expressed and pathway gene sequences contained in the clones listed in Table 2, above, as deposited with the NRRL, or contained in the coding regions of the genes to which DNA sequences disclosed herein (in FIGS. 8 (SEQ ID NO. 1), 12 (SEQ ID NO. 2), 15 (SEQ ID NO. 3), 18 (SEQ ID NO. 4), 22 (SEQ ID NO. 5), 28 (SEQ ID NO. 38), 31 (SEQ ID NO. 7), and 35 (SEQ ID NOS. 36-37)) or contained in the clones, listed in Table 2, as deposited with the NRRL, belong, for example.

Page 107, line 23, through Page 108, line 2:

First strand cDNA synthesis: For each RNA sample duplicate reactions were carried out in parallel. 400 ng RNA plus DEPC H_20 in a total volume of 10 µl were added to 4 µl $T_{11}XX$ (SEQ ID NO. 31) reverse primer (10 µM) (Operon). The specific primers used in each experiment are provided in the Description of the Figures in Section 4, above. The mixture was incubated at 70°C for 5 min. to denature the RNA and then placed at r.t. 26 µl of reaction mix containing the following components was added to each denatured RNA/primer sample: 8 µl 5x First Strand Buffer (Gibco/BRL, Gaithersburg, MD), 4 µl O.1M DTT (Gibco/BRL), 2 µl RNAse inhibitor (40 units/µl) (Boehringer Mannheim), 4 µl 200 µM dNTP mix, 6 µl H_20, 2 µl Superscript reverse transcriptase (200 units/µl) (Gibco/BRL). The reactions were mixed gently and incubated for 30 min. at 42°C. 60 µl of H_20 (final volume = 100 µl) were then added and the samples were denatured for 5 min. at 85°C and stored at -20°C.

Page 119, lines 14 through 24:

Lipoproteins were prepared as described, above, in section 6.1.1. Differential display, Northern analysis, RT-PCR, subcloning, and DNA sequencing were carried out as described, above, in Section 6.1.2. For differential display, the primers used were $T_{11}CC$ (reverse) and OPE4 (forward), consisting of 5'GTGACATGCC3' (SEQ ID NO. 33). For RT-PCR, the first strand cDNA was primed with $T_{11}CC$ (SEQ ID NO. 32), and PCR reactions were carried out with rfhma15 primers (for-catgcctgtagaaaaaggtt (SEQ ID NO. 34)/rev-cttcatagaatctaagccta) (SEQ ID NO. 35), and mouse γactin primers (for-cctgatagatgggcactgtgt/rev-gaacacggcattgtcactaact).

Page 124, line 8 through page 125, line 21:

8.2. RESULTS

HUVEC's were activated with 10 units/ml IL-1β for 1 or 6 hours and compared to resting HUVEC's using differential display. As shown in FIG.5, a band marked rchd005 is present in lanes 11 and 12 (IL-1, 6 hr.) but not in lanes 9 and 10 (control), or lanes 7 and 8 (IL-1, 1 hr.). This band, rchd005, was isolated and subcloned and sequenced. When a probe prepared form this band was used to screen a Northern blot, expression was seen at 6 hr., but not at 1 hr. or in the control (FIG.6). However, when this same probe was hybridized to a Northern blot prepared from shear stressed RNA, according to Paradigm D described in Section 9, below, a different pattern of up-regulation was also seen (FIG.7). Expression was up at 1 hr. and then nearly disappeared by 6 hr. Amplified rchd005 DNA was subcloned and sequenced. Sequence analysis revealed an approximately 360 bp insert (FIG.8) (SEQ ID NO. 1) with 70% sequence similarity to a cloned shark gene called bumetanide-sensitive Na-K-Cl cotransport protein.

Another IL-1 inducible band, rchd024, is shown in FIG.9. Northern analysis on IL-1 up-regulated RNA reveals a 10 kb message present at 6 hr. (FIG.10) that also shows a low level of up-regulation under shear stress at 6 hr. (FIG.11). The DNA sequence was obtained from subclones of amplified DNA (FIG.12) (SEQ ID NO. 2). Database searching revealed no significant sequence similarities. A PCR amplification experiment determined that the rchd024 gene is located on human chromosome 4.

Band rchd032 was isolated on the basis of its differentially increased expression after 6 hr. treatment with IL-1 (FIG.13), which was confirmed by RT-PCR analysis (FIG.14). Amplified rchd032 sequences were subcloned and sequenced (FIG.15) (SEQ ID NO. 3). No significant homology to any known gene was found.